18th Symposium on Purine and Pyrimidine Metabolism in Man

12th-14th of June 2019, Lyon, France

The Purine and Pyrimidine Society

Physicians and Scientists Investigating Purines and Pyrimidines in Man

General information and program

S5.4	9.55-10.10	Chiara Rampazzo	SAMHD1 deficiency affects DNA replication fidelity and telomere homeostasis in human fibroblasts
S5.5	10.10-10.30	Miriam Yagüe Capilla	Deciphering the role of HD52, a mitochondrial nucleotidase essential for pyrimidine homeostasis in *Trypanosoma brucei*
S5.6	10.30-10.45	Ken Okamoto	Effect of xanthine oxidoreductase inhibitors on purine metabolism in mouse brain under hypoxic environment
S5.7	10.45-11.00	Paulina Mierzejewska	Ecto-5'-nucleotidase (CD73) deficiency in mice leads to age dependent impaired L-arginine metabolism and endothelial dysfunction
11.00-11.35		Coffee break and Poster session 2	
11.35-13.00		Session 6 **Inborn errors of purine and pyrimidine metabolism 1** Chairs: Yolanda Cámara & Sylvain Latour	
S6.1	11.35-12.00	Aida Mata-Ventosa	Deficiency of perforin and hCNT1, a novel inborn error of pyrimidine metabolism, associated with a rapidly developing lethal phenotype due to multi-organ failure
S6.2	12.00-12.20	Jonathan Shintaku	Ribonucleotide Reductase Subunit RRM1 is Required for Mitochondrial DNA Maintenance via Regulation of dNTP and rNTP Pools
S6.3	12.20-12.40	Norbert Minet	Characterization and complementation of cellular models of CTPS1 and CTPS2 deficiencies
S6.4	12.40-13.00	Emmanuel Martin	A homozygous hypomorphic mutation is responsible for CTPS1 immunodeficiency : immunological and molecular characterization from a cohort study
13.00-14.30		Lunch and Poster session 2	
14.30-16.00		Session 7 **Purines and pyrimidines in cancer 2** Chairs: Tormod Karlsen Bjånes & Takahiro Yamauchi	
S7.1	14.30-14.50	Btissame El Hassouni	Acquired resistance to Fluorocyclopentenylcytosine (RX-3117) in non-small lung cancer cells is related to a decrease of active RX-3117 nucleotides
S7.2	14.50-15.10	Claus Desler Madsen	The importance of nucleotide metabolism for successful immunotherapy for cancer
S7.3	15.10-15.25	Octavia Cadassou	What roles for the 5'-nucleotidases cN-II and CD73 in the interplay between the cancer cell and its innate immune microenvironment?
S7.4	15.25-15.40	Mihoko Morita	How the combination of 6-mercaptopurine with febuxostat affects xanthine oxidase activity in vitro
S7.5	15.40-16.00	Fiona McKissock	Novel ProTide NUC-3373: a potent inhibitor of thymidylate synthase
17.00-18.30		Sightseeing in the old city of Lyon	
19.30-21.00		PPS Board Meeting	
Friday June 14th 2019		*The Auditorium, IARC*	
8.30-9.50		Session 8 **Inborn errors of purine and pyrimidine metabolism 2** Chairs: Sandra Pérez-Torras & André van Kuilenburg	
S8.1	8.30-8.45	Michio Hirano	Deoxynucleoside Therapy for Mitochondrial DNA Depletion
S8.2	8.45-9.00	Cora Blázquez-Bermejo	Age-related metabolic changes limit efficacy of deoxynucleoside-based therapy in TK2-deficient mice
S8.3	9.00-9.15	Olga Součková	Preparation of standards for LC-MS/MS detection of various metabolites of the *de novo* purine synthesis and their

Abstract S5.4

Deciphering the role of HD52, a mitochondrial nucleotidase essential for pyrimidine homeostasis in *Trypanosoma brucei*

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Objectives: We aimed to elucidate the role of TbHD52, a nucleotidase that belongs to the SAMHD1 family, in dTTP biosynthesis and pyrimidine homeostasis in *Trypanosoma brucei*.

Methods: Intracellular localization of TbHD52 was determined by immunofluorescence studies. TbHD52 knock-out cell lines were generated upon thymidine supplementation to further establish the impact of TbHD52 on cell viability, cell cycle progression and pyrimidine homeostasis. Several cell lines overexpressing enzymes involved in pyrimidine metabolism, such as hDCTD or TbCDA, in a *TbHD52*-dKO background were also generated for a more comprehensive study. Additionally, metabolomics studies were performed in order to identify global disturbances, specifically in dNTP homeostasis, in the absence of TbHD52.

Results: TbHD52 is an exclusive mitochondrial nucleotidase essential for *Trypanosoma brucei* viability, as knock-out cells are pyrimidine auxotrophs. Additionally, our findings show that the lack of HD52 can be counteracted by the expression of enzymes involved in dUMP formation. In the absence of exogenous thymidine or deoxyuridine supplementation, *TbHD52*-dKO cells show strong defects in cell cycle progression and nuclei and kinetoplast segregation. Furthermore, the metabolomics profile was severely perturbed, affecting profoundly cytosine- and thymidine-derived metabolites.

Conclusions: TbHD52 is an essential enzyme in *Trypanosoma brucei* and our results suggest that it plays a key role providing mitochondrial deoxycytidine and thymidine for dTTP biosynthesis via TK salvage. Thus, our findings firmly support that TbHD52 is a valuable drug target against African trypanosomiasis.

Relevant references:

- Valente, M., J. Timm, *et al*. (2016). "Cell cycle regulation and novel structural features of thymidine kinase, an essential enzyme in *Trypanosoma brucei*." Mol Microbiol **102**(3): 365-385.
- Leija, C., F. Rijo-Ferreira, *et al*. (2016). "Pyrimidine salvage enzymes are essential for *de novo* biosynthesis of deoxypyrimidine nucleotides in *Trypanosoma brucei*." PLoS Pathog **12**(11): e1006010.
- Ji, X., C. Tang, *et al*. (2014). "Structural basis of cellular dNTP regulation by SAMHD1." Proc Natl Acad Sci U S A **111**(41): E4305-14.
- St Gelais, C., S. de Silva, *et al*. (2012). "SAMHD1 restricts HIV-1 infection in dendritic cells (DCs) by dNTP depletion, but its expression in DCs and primary CD4+ T-lymphocytes cannot be upregulated by interferons." Retrovirology **9**: 105.

KEYWORDS: *Trypanosoma brucei*, SAMHD1, pyrimidines, nucleotidase